Modeling Glioblastoma heterogeneity using 3D cerebral organoids

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Abstract:

Glioblastoma (GBM) is an aggressive and still incurable brain tumor. Recent work demonstrates that GBM is profoundly heterogeneous at the cellular level and displays strong patient-specific effects, underscoring the need for individualized approaches to model its invasive behavior. Brain organoid–based 3D co-culture systems (GLICOs) have recently emerged as valuable in vitro models that better recapitulate tumor growth and dynamics and interaction with the neural microenvironment. In this work, we established the patient-specific GLICOs model of GBM by co-culturing healthy brain organoids derived from human embryonic H9 stem cells with different patient-derived GBM stem cell lines (GSC7 and L1312).

In the first part of this work, we will describe a pilot study for the generation of GLICO using the GSC7 line. 20-day cerebral organoids were co-cultured with either 20,000 or 100,000 GFP-labelled GSC7 cells and monitored for one week by fluorescence microscopy to assess tumor cell invasion and migration. At the endpoint, immunofluorescence analyses were performed to evaluate the presence of GFP-GSC7 cells within the organoids, and their proliferative state. We found that GSC engraftment was successful in both cases. However, the highest number of GSCs spread throughout the entire surface of the organoid, impairing the growth of organoids. In contrast to this, GCS at 20,000 cells formed well-separated engraftment sites, leading to less pronounced effects on organoid growth.

In the second part of the work, we utilized another patient-derived GBM line, L1312, marked by high cellular and molecular heterogeneity. Building on the previously established protocol for GSC7, we tested a wider range of cell-seeding densities (2,000, 5,000, 10,000, and 20,000 GFP-labelled L1312 cells) and further extended the co-culture protocol of two weeks to allow more time for GBM cells to invade and migrate within the organoids. Preliminary observations suggest that 20,000 cells is an excessive dose, as L1312 cells surround the organoids after only one day of co-culture.

Although experiments are still ongoing, these preliminary observations suggest a striking difference in organoid engraftment capacity between the GSC7 and L1312 GSCs, in line with the extreme cellular heterogeneity reported in the literature. These findings support the use of patient-specific GLICO models to study the tumor microenvironment and for individualized treatments.